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Larvicidal activity of *Hypoestes forskaolii* (Vahl) R. Br root extracts against *Anopheles gambiae* Giless. s, *Aedes aegypti* L, and *Culex quinquefasciatus Say*

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Aim: This study aimed to evaluate larvicidal activity of *Hypoestes forskaolii* R. Br root extract against 3rd instar *Anopheles gambiae*, *Aedes aegypti*, *and Culex quinquefasciatus*.

Methods: A protocol developed by the World Health Organization was adopted, with minor modification using chloroform and methanol extracts with concentrations ranging from 25–750 µg/mL.

Results: The *H. forskaolii* chloroform extract exhibited very high larvicidal activity after 72 hours of exposure, with LC_{50} 2.0322, 3.8989, 6.0004 µg/mL against *A. gambiae, A. aegypti*, and *C. quinquefasciatus*, respectively.

Conclusion: The larvicidal activity of *H. forskaolii* is reported for the first time in this paper. The effectiveness of *H. forskaolii* chloroform extract warrants further research to develop botanical mosquito repellants from this source.

Keywords: *Hypoestes forskaolii*, LC_{50}, *Anopheles gambiae*, *Aedes aegypti*, *Culex quinquefasciatus*

Video abstract

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Introduction

Mosquitoes are a potential vector of several tropical diseases, including numerous viral diseases: of 3,000 species existing, 100 are known to be vectors.[1] It has been reported that mosquitoes are more effective in disease transmission than any other arthropods, and thus are regarded public enemy number one, as reported by the World Health Organization (WHO).[2] Mosquitoes are known to transmit such diseases as malaria, dengue fever, chikungunya, Rift Valley fever, filariasis, West Nile fever and Japanese encephalitis.[3,4] It is estimated that more than 700 million people are infected with mosquito-transmitted diseases annually, which leads to death, poverty, and social and economic disturbances.[5] Synthetic chemical pesticides have been used for a long time in controlling mosquitoes, but their arbitrary use has given rise to known and serious problems, including genetic resistance, increasing cost of application, hazards from handling, and environmental pollution.[6,7] The search for effective and biodegradable pesticides, including mosquito repellents, is of paramount importance. One of the potential source is plants that are known to be used by communities in the management of insects.

In Tanzania, *H. forskaolii* is used for the management of houseflies, especially among pastoralist communities. The concoction from the roots of this pant is mixed with milk and placed in an open area. Milk is used, as it attracts houseflies and cockroach especially. Insects that feed on the product die instantly as they feed. The

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remarkable activity of *H. forskaolii* against houseflies and cockroaches prompted our research group to investigate the lavicidal activity of *H. forskaolii* against third-instar *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus*.

H. forskaolii is an annual or perennial herb that grows up to 1 m tall, with its stem and leaves being nearly glabrous. It has pale-pink or white flowers. It is a polymorphic species found in most habitats. It is most common in open woodland and wooded grassland on sandy soils or rocky slopes and in disturbed areas, such as roadsides. It also occurs in riverine areas and open forest. The plant species is widespread in tropical and southern Africa from Senegal to Somalia, south of Namibia, and South Africa. It extends to the Saharan highlands, the Arabian Peninsula, and Madagascar. Musayeib et al reported antiprotozoal activity of methanolic extracts against *Plasmodium falciparum, Leishmania infantum, Trypanosoma cruzi*, and *Trypanosoma brucei*.[8] This paper thus report the antilarvicidal activity of *H. forskaolii* chloroform and methanolic extracts.

Methods

Chemicals and mosquito larvae

Chloroform, methanol and dimethyl sulfoxide (DMSO) were purchased from Avantor Performance Materials India. The third-instar larvae of *A. gambiae, A. aegypti and C. quinquefasciatus* were obtained and reared at the Tropical Pesticides Research Instutute, Arusha, Tanzania.

Collection of plant roots and preparation of extracts

H. forskaolii was identified by Dr Ephraim Njau of the National Herbarium, Tropical Pesticide Research Institute at the collection site (Hanang district in Manyara region, Tanzania). The plant specimen is kept at the Nelson Mandela Institution of Science and Technology, coded as HF1423. Roots were chopped, washed, blended, and sequentially macerated using chloroform (analytical grade) and methanol (analytical grade) for 72 hours. Solvents were removed through vacuum with a rotary evaporator (Heidolph, Germany). Methanolic and chloroform extracts were kept in the refrigerator at −20°C until testing.

Larvicidal activity

The WHO protocol of 1996 year was adopted for larvicidal assays, with minor modifications.[9] Larvae of *A. aegypti* and *C. quinquefasciatus* were fed with dog biscuits, while those of *A. gambiae* were fed with tetramine during experiment. Stock solutions for methanol and chloroform extracts (500 mg/mL) were established by dissolving 500 mg crude extract in 5 mL DMSO. With serial dilution, concentrations of 25, 50, 100, 200, and 750 µg/mL were prepared from stock solution. Distilled water was used in the serial dilution. Ten late third–instar mosquito larvae were introduced into the test solution and mortality observed and recorded after 24, 36, and 72 hours. Cups with ten mosquito larvae, distilled water, and 0.5 µg/mL DMSO were taken as negative controls. All experiments were done in triplicate under controlled temperature of 25°C±2°C and relative humidity of 75%−85%. Dead larvae were identified by lack of mobiility and not being able to reach the water's surface.

Statistical analysis

FigP software (Biosoft, Cambridge, UK) was used for analysis, and mean percentage mortality was plotted against logarithms of concentrations. For regression equations, LC_{50}, CIs and regression coefficients were calculated.

Results

Larvicidal bioassay results performed on early-third instars of *A. aegypti, A. gambiae*, and *C. quinquefasciatus* with chloroform and methanolic root extracts of *H. forskaolii* are presented in Tables 1–3, respectively. Mosquito larvae were exposed to extracts prepared in DMSO at 25–750 µg/mL and mortality recorded after 24, 36, and 72 hours' exposure. Since the WHO has not established standard criteria for determining the larvicidal activity of natural products, several authors have developed individual sets of criteria to characterize the potency of mosquito larvicides developed from natural products.[10]

According to Komalamisra et al, larvicidal activity of the plant extract is consideredinactive when LC_{50} is >750 µg/mL, weakly effective if LC_{50} is 200–750 µg/mL, moderate if LC_{50} is 100–200 µg/mL, effective if LC_{50} is 50–100 µg/mL, and highly effective if LC_{50} is <50 µg/mL.[11] Results from this study displayed larvicidal activity for *H. forskaolii* against three species of mosquito tested, giving LC_{50} values of 220.4789–3.8989 µg/mL for *A. aegypti* (Table 1), 69.6596–2.0322 µg/mL for *A. gambiae* (Table 2), and 177.5595–6.0004 µg/mL for *C. quinquefasciatus* (Table 3). Chloroform and methanolic extracts were highly effective after 72 hours' exposure, and this showed the extracts were remarkably effective in controlling the mosquito larvae tested. The activity was species-specific, which clearly revealed that chloroform

Table 1 Larvicidal activity of Hypoestes *forskaolii* root extracts against *Aedes aegypti*

Extract code	Time	LC_{50} (µg/mL)	95% CI	R^2	Regression equation
HFCE	24 hours	154.6019	1,706.3408–14.0076	0.938	$y=10.57\log x+26.86$
	36 hours	15.0053	110.7175–2.0336	0.93	$y=10.44\log x+37.72$
	72 hours	3.8989	22.1742–0.6856	0.87	$y=12.15\log x+42.82$
HFME	24 hours	220.4789	904.4034–53.7492	0.994	$y=18.77\log x+6.015$
	36 hours	56.3484	226.2731–14.0323	0.946	$y=17.02\log x+20.20$
	72 hours	11.5432	54.3812–2.4502	0.96	$y=14.28\log x+34.83$
Control	NM	–	–	–	–

Abbreviations: HFCE, *H. forskaolii* chloroform extract; HFME, *H. forskaolii* methanolic extract; NM, no mortality.

Table 2 Larvicidal activity of Hypoestes *forskaolii* root extracts against *Anopheles gambiae*

Extract code	Time	LC_{50} (µg/mL)	95% CI	R^2	Regression equation
HFCE	24 hours	69.6596	330.1227–14.6989	0.853	$y=15.03\log x+22.30$
	36 hours	8.8111	33.1905–2.3391	0.954	$y=16.19\log x+34.70$
	72 hours	2.0322	6.6260–0.6233	0.866	$y=16.82\log x+44.82$
HFME	24 hours	37.1001	159.9947–8.6029	0.984	$y=16.00\log x+24.89$
	36 hours	7.4977	25.1042–2.2393	0.940	$y=17.43\log x+34.75$
	72 hours	9.5728	15.22–1.5400	0.973	$y=8.965\log x+68.02$
Control	NM	–	–	–	–

Abbreviations: HFCE, *H. forskaolii* chloroform extract; HFME, *H. forskaolii* methanolic extract; NM, no mortality.

Table 3 Larvicidal activity of *Hypoestes forskaolii* root extracts against *Culex quinquefasciatus*

Extract code	Time	LC_{50} (µg/mL)	95% CI	R^2	Regression equation
HFCE	24 hours	177.5595	1661.1320–18.9794	0.856	$y=11.43\log x+24.29$
	36 hours	18.0962	97.3554–3.3637	0.933	$y=13.51\log x+33.01$
	72 hours	6.0004	27.4143–1.3133	0.851	$y=13.93\log x+39.16$
HFME	24 hours	137.7328	530.6684–35.7471	0.96	$y=18.70\log x+10.55$
	36 hours	31.7442	112.8697–8.9271	0.97	$y=18.02\log x+22.94$
	72 hours	6.4358	23.0496–1.7961	0.972	$y=16.51\log x+36.65$
Control	NM	–	–	–	–

Abbreviations: HFCE, *H. forskaolii* chloroform extract; HFME, *H. forskaolii* methanolic extract; NM, no mortality.

extract had higher larvicidal activity: LC_{50} 3.8989 µg/mL against *A. aegypti*, µg/mL 2.0322 against A. *gambiae*, and 6.004 µg/mL against *C. quinquefasciatus* after 72 hours' exposure. The methanolic extract had an LC_{50} of 11.5432 µg/mL against *A. aegypti*, 9.5728 µg/mL against *A. gambiae*, and 6.4358 against *C. quinquefasciatus* after 72 hours' exposure. The results also showed effective, moderate, and weakly effective larvicidal activity for both chloroform and methanolic extracts after 24 hours' exposure. Chloroform extracts had an LC_{50} of 154.6019 µg/mL against *A. aegypti*, 69.6596 µg/mL against *A. gambiae*, and 177.5595 µg/mL against *C. quinquefasciatus*. Methanolic extracts also possessed activity: LC_{50} of 220.4789 µg/mL against *A. aegypti*, 37.1001 µg/mL against *A. gambiae*, and 137.7228 against *C. quinquefasciatus*. Larvicidal effects of *H. forskaolii* root extract were all <750 µg/mL, which justifies its use in managing the mosquito larvae tested.

Discussion

Mosquitoes in the larval stage are attractive targets for pesticides, because their breeding sites in stagnant water can be easily accessed, but the use of chemical pesticides in water sources introduces more risks to humans and the environment.[12,13] Natural pesticides derived from plants are thus promising tools, especially for managing mosquito larvae.[14–16] *H. forskaolii* has been used to manage insect vectors and handling insect vector–borne diseases in Tanzania.[17] The latter study revealed larvicidal activity of

H. forskaolii against *A. aegypti, A. gambiae*, and *C. quinquefasciatus*. Mortality was up to 50%, with LC_{50} of 220.478–2.0322 µg/mL. *H. forskaolii* chloroform extract demonstrated the highest larvicidal activity, with LC_{50} of 3.8989, 2.0322. and 6.004 µg/mL against *A. aegypti, A. gambiae*, and *C. quinquefasciatus*, respectively. These findings of the lavicidal activity of *H. forskaolii* root extracts suggest the use of this plant in the management of mosquitoe larvae.

Winisia et al reported on larvicidal activity of fruits and leaves of *Clausena anisata* growing in Tanzania. *C. anisata* ethyl acetate and methanolic leaf extracts exhibited remarkable larvicidal activity, with LC_{50} of 0.0977 and 0.9362 µg/mL.[18] This information qualifies the potential of Tanzania plants in the management of mosquitoes and thus mosquito-borne diseases.

Gas chromatography–mass spectrometry was used to analyze *H. forskaolii* chloroform root extract, wherein 102 secondary metabolites belonging to sesquiterpenes, diertepenes, fatty acids and alkane were identified. Of the phytochemical compounds identified caryophyllene and caryophyllene oxide have been reported to exert larvicidal activity.[19,20]

Mosquito management has been exercised through tremoval of mosquito habitats, use of structural barriers, control of mosquitoes at the larval stage, and control of adult mosquitoes. In some cases, an integrated mosquito-control strategy has been used. Each tactic has its own advantages and disadvantages. Focusing mosquito-reduction efforts on the larval stage has the advantage of controlling the vector prior to dispersal or acquisition of the disease and interrupting the life cycle before it can cause harm. Although a botanical larvicidal agent has not yet impacted the market, there are a number of chemicals available to target mosquito larvae, including such organophophates as temephos and insect growth regulators like methoprene,[21] although resistance has been found to each of these in the field.-[22,23] Resistance of mosquito larvae to available larvicidal agents has prompted the intensity of the search for agents. The findings presented in this paper throw light on the possibility of developing botanical larvicidal agents from *H. forskaolii*.

Conclusion

The results clearly reveal that both chloroform and methanol root extract of *H. forskaolii* are potential sources of larvicidal agents against *A. aegypti, A. gambiae*, and *C. quinquefasciatus.*

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Abou-Enaga ZS. Insecticidal bioactivity of ecofrinedly plant origin chemicals against *Culex pipiens* and *Aedes aegypti* (Diptera: culicidae). *J Entomol Zool Stud*. 2014;2(6):340–347.
2. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. *Indian J Med Res*. 2012;135:581–598.
3. Dua VK, Pandey AC, Dash AP. Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. *Indiana J Med Res*. 2010;131:434–439.
4. Ashwini U, Asha S. Larvicidal activity of natural products against mosquito species. *A review. Int J Chem Tech Res*. 2017;10(5):875–878.
5. Mavundza EJ, Maharaj R, Chukwujekwu JC, Finnie JF, Staden JV. Screening for adulticidal activity against *Anopheles arabiensis* in ten plants used as mosquito repellent in South Africa. *Malar J*. 2014;13:173. doi:10.1186/1475-2875-13-173
6. Adeyemi MMH. Potential of secondary metabolites in plant material as detergents against insect pests: A review. *Afr J Pure Appl Chem*. 2010;4(11):243–246.
7. Lee SE. Mosquito larvicidal activity of pipernonaline, A piperidine alkaloid derived from long pepper piper longum. *J Am Mosq Control Assoc*. 2000;16(3):245–247.
8. Report of the WHO informal consultation on the Evaluation and Testing of Insecticides, WHO pesticides evaluation scheme (WHOPES) world health organization division of control of tropical diseases (CTD), WHO, Geneva; 7–11 October 1996, CTD/WHOPES/IC/96.1
9. Musayeib NMA, Ra M, Ga M, Srm I, Maes L. Hypoestenonols A and B, new fusicocane diterpenes from Hypoestes forskalei. *Phytochem Lett*. 2014;10(1):23–27. doi:10.1016/j.phytol.2014.06.020
10. Dias CN, Alves LP, Rodrigues KA, et al. Chemical composition and larvicidal activity of essential oils extracted from Brazilian Legal Amazon plants against *Aedes aegypti* L. (Diptera: culicidae). *Evid Based Complement Alternat Med*. 2015;2015:490765.
11. Komalamisra N, Trongtokit Y, Rongsriyam Y, Apiwathnasorn C. Screening for larvicidal activity in some Thai plants against four mosquito vector species. *Southeast Asian J Trop Med Public Health*. 2005;36(6):1412–1422.
12. Subramanian J, Kovendan K, Kumar PM, Murugan K, Walton W. Mosquito larvicidal activity of Aloe vera (Family: liliaceae) leaf extract and *Bacillus sphaericus*, against Chikungunya vector, Aedes aegypti. *Saudi J Biol Sci*. 2012;19:503–509. doi:10.1016/j.sjbs.2012.07.003
13. Bagavan A, Rahuman AA. Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors. *Asian Pac J Trop Med*. 2011;4:29–34. doi:10.1016/S1995-7645(11)60027-8

14. Warikoo R, Ray A, Sandhu JK, Samal R, Wahab N, Kumar S. Larvicidal and irritant activities of hexane leaf extracts of tropical biomedicine. *Asian Pac J Trop Biomed*. 2012;2(2):152–155. doi:10.1016/S2221-1691(11)60211-6
15. Mukhatar MU, Mushtag S, Arkam A, Zaki B, Hammad M, Bhatti A. Laboratory study on larvicidal activity of different plant extracts against *Aedes aegypti*. *Int J Mosquito Res*. 2015;2(3):127–130.
16. Hemalatha P, Elumalai D, Janaki A, et al. Larvicidal activity of Lantana camara aculeate against three important mosquito species. *J Entomol Zool Stud*. 2015;3(1):174–181.
17. Asnake S, Teklehaymanot T, Hymete A, Erko B, Giday M. Survey of medicinal plants used to treat malaria by Sidama people of Boricha district, Sidama zone, south region of Ethiopia. *Evid Based Complement Alternat Med*. 2016;2016:9690164.
18. Makirita W, Chauka L, Chacha M. Larvicidal activity of *Clausena anisata* fruits and leaves extracts against *Anopheles gambiae Giless.s, Culex quinquefasciatus Say* and *Aedes aegyptiae*. *Spatula DD*. 2015;5(3):147–153. doi:10.5455/spatula.20151118060743
19. Xc L, Liu QY, Zhou L, Zl L. Larvicidal activity of *Isodon japonicus* var. *glaucocalyx* (Maxim.) H. W .Li essential Oil to *Aedes aegypti* L. and its chemical composition. *Trop J Pharm Res*. 2014;13(9):1471–1476. doi:10.4314/tjpr.v13i9.13
20. Mathew J, Thoppil JE. Chemical composition and mosquito larvicidal activities of *Salvia* essential oils. *Pharm Biol*. 2011;49(5):456–463. doi:10.3109/13880209.2010.523427
21. Rose RI. Pesticides and public health: integrated methods of mosquito management. *Emerging Infect Dis*. 2001;7:17–23. doi:10.3201/eid0701.010103
22. Dame D, Wichterman G, Hornby J. Mosquito (*Aedes taeniorhynchus*) resistance to methoprene in an isolated habitat. *J Am Mosquito Control Assoc*. 1998;14:200–203.
23. Raymond M, Berticat C, Weill M, Pasteur N, Chevillon C. Insecticide resistance in the mosquito *Culex pipiens*: what have we learned about adaptation? *Genetica*. 2001;112:287–296.